Correlation between gene polymorphism rs7903146 and diabetes mellitus in Iraqi population

A Research
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قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلِمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم

سورة البقرة: الآية 32
Dedication

To who was present with me at all times in my heart and mind to prophet of peace…

“Mohammed”

Peace and prayer be on him and his purified family.

To my great family who encouraged me

To all my friends

Zahraa Ali Mohammed and Zahraa Ali Mahdi
Acknowledgments

First of all, praise is to “Allah”, who enabled us to overcome all the difficulties that were associated with this work till I brought it to the present state. Peace and prayer be on the most honorable Mohammed and his purified family.

I would like to express my sincere gratitude to my supervisor Dr. Mohammed Al-Askeri for his encouragement and useful advice that she provided during this study. Special thanks go to the Head of the Department Dr. Nazar Hamza for his encouragement, and advice. We would like to thank Dean of College of Biotechnology/ University of AL-Qadisyah and the staff of Biotechnology Department with our gratitude.

Last, but not least, Great gratitude and deep appreciation go to our families, who were always listening to our complaints, frustration, and for believing in our. We are the person who we are today because of you.

Thank you.
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1. Introduction

Diabetes mellitus (DM) is a serious medical problem affecting millions of peoples worldwide, and has a great socio-economic impacts(1).

The prevalence of type 2 diabetes (T2D) is increasing in an alarming rate(2-4) Population ageing and obesity are considered the main causes of diabetes (5). Although the risk of developing the disease is also strongly influenced by inheritance in which Genetic susceptibility to T2D is believed to be polygenic (6, 7)as it has been indentified transcription factor-7-like 2 (TCF7/L2) as susceptible gene (8, 9).Genetic variants in the gene encoding this transcription factor have been consistently associated with T2D and impaired insulin secretion Among them, the rs7903146 T allele is probably the best marker to evaluate the effect of this gene on T2D risk(4).

This allele increases the risk of T2D by 1.45 in heterozygous and by 2.41 in homozygous .All these findings have been replicated in populations of different ethnic descent including caucasian Europeans Japanese and Indian people ,Latin Americans and West Africans (6, 10, 11) , representing the main ethnical groups with a high prevalence of T2D(12).

ELMO1 is a soluble cytoplasmic protein that functionally cooperates with CRKII and dedicator of cytokinesis 180 (DOCK180) to mediate cytoskeletal rearrangements during phagocytosis of apoptotic cells and cell motility in mammalian cells (Gumienny et al., 2001). Functional studies of ELMO1 expression reveal that increased levels were observed in COS cells cultured at high glucose concentration, loss of cell adhesion properties and enhanced synthesis of collagen and fibronectin in ELMO1 transfected cells suggest a pathological role in kidney disease (Shimazaki et al., 2005; Shimazaki
et al., 2006). This study aims at exploring any possible correlation between diabetes mellitus and gene polymorphism rs7903146.

2. Materials and Methods

2.1 Materials

2.1.1 Equipment and Apparatus

Different equipment and apparatuses have been used throughout the study as shown in table 1

Table 1: Equipment used in the study

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoclave</td>
<td>Gallenkamp (England)</td>
</tr>
<tr>
<td>Cooled centrifuge</td>
<td>Labnet (USA)</td>
</tr>
<tr>
<td>Thermocycler</td>
<td>Labnet (USA)</td>
</tr>
<tr>
<td>Distillatory unit</td>
<td>Kent (England)</td>
</tr>
<tr>
<td>Hot plate magnetic stirrer</td>
<td>Stuart scientific (U.K.)</td>
</tr>
<tr>
<td>Sensitive balance</td>
<td>Sartorius (Germany)</td>
</tr>
<tr>
<td>Spectrophotometer</td>
<td>Labnet(USA)</td>
</tr>
<tr>
<td>Vortex mixer</td>
<td>Buchi (Switzerland)</td>
</tr>
<tr>
<td>DNA –Gel Electrophoresis</td>
<td>Labnet ( USA)</td>
</tr>
<tr>
<td>Micropipettes</td>
<td>Witeg ( USA)</td>
</tr>
</tbody>
</table>
### 2.1.2 Chemicals and buffers

#### 2.1.2.1 TBE buffer 5X (Maniatis et al., 1982)

It is composed of:

- Tris-Base: 54 g
- Boric acid: 27.5 gm
- EDTA 0.5M (pH 8): 20 ml

The volume was brought to up 1 L and autoclaved.

#### 2.1.2.2 Ethidium bromide solution (10 mg/ml) (Maniatis et al., 1982)

Ethidium bromide (0.1 g) was dissolved in 10 ml of D.W and stirred with a magnetic stirrer for six hours to ensure the complete dissolving, then it filtrated and stored in a dark bottle, wrapped with aluminum foil at 4°C.

#### 2.1.2.3 Agarose gel

Agarose 1% concentration was used, dissolved in TBE 1X using hotplate.
2.2 Methods

2.2.1 Study individuals
This study was carried out on 40 Iraqi individuals, aged between (14-69) years represented by 20 patients who admitted to Al-Diwaniyha teaching hospital and 20 healthy controls. Blood samples were collected from subjects. About three milliliters of blood withdrawal from each subject and placed into Ethylenediaminetetraacetic acid (EDTA)-tubes then transferred to the laboratory in cooling conditions in less than one hour.

2.2.2 DNA Extraction
DNA was isolated from peripheral blood by means of FavorPrep Blood Genomic DNA Extraction Mini Kit (South Korea) according to the manufacturer’s instructions at Department of Medical Biotechnology / College of Biotechnology / University of Al-Qadisiyha and stored at -20°C for Polymerase Chain reaction.

2.2.3 Genotyping
Genotyping was took place on cycler machine (LABNET) using primers table (2). Amplification conditions were 35 cycles of 94°C / four minutes, 94°C / 30 seconds, 63°C / one minute, and 72°C / two minutes with a final extension step of 72°C / eight minutes, PCR products were run on 1.8% agarose gel and stained with Ethidium bromide then analyzed using UV transilluminator, standard DNA ladder 100bp (Bioneer, South Korea) was used.

DNA amplifications were repeated three times using the same conditions to confirm the results with negative controls. Product sizes in order to check the variant rs1345365 polymorphism are 629 bp for two outer primers (control bands), 284 bp for G allele and 461 bp for A allele according to (13).
Table (2): Specific primers applied for polymorphism determination of ELMO1 gene

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward inner</td>
<td>5’ GCCACCTTCTTCCCCTACAACATTGA 3’</td>
</tr>
<tr>
<td>Reverse inner</td>
<td>5’ GCCAGTGAGAGAGTAATACTATTACGTTCT3’</td>
</tr>
<tr>
<td>Forward outer</td>
<td>5’ TGCCATAGGTACTGCTTCTCTGAGT 3’</td>
</tr>
<tr>
<td>Reverse outer</td>
<td>5’ CTGAAGTCTAGTAAGAGCTCAAGGTCAGT 3’</td>
</tr>
</tbody>
</table>
3. Results and discussion
Specific primers were used in an attempt to detect a possible genetic polymorphism between patients diagnosed with diabetes mellitus in Al-Qadisiyha. There are many scientific methods and protocols were applying by many researcher and laboratory workers to detect and analyze the mutations that are related to diabetes mellitus type 2. Tetra-ARMS PCR technique is considered as one of the best technique due to its easy and inexpensive to be compared with the other technique in which we prefer to use it in this study.

Figure 1: 1% Agarose electrophoresis of PCR product 5v/cm, lane1, DNA ladder 100 bp; lane2-3, patients; lane 4-8: healthy individuals
Tetra-ARMS PCR technique that applied in this study revealed presence of single nucleotide polymorphism figure (1-2)

Figure 2: 1% Agarose electrophoresis of PCR product 5v/cm, lane1, DNA ladder 100 bp; lane2-3, patients; lane 4-8: healthy individuals

ELMO1 is a soluble cytoplasmic protein that functionally cooperates with CRKII and dedicator of cytokinesis 180 (DOCK180) to mediate cytoskeletal rearrangements during phagocytosis of apoptotic cells and cell motility in mammalian cells (14).

The results showed the allelic and genotyping of the individuals covered by this study as it appears in table (3).
Table 3: rs7903146 genotypes in patients and healthy individuals

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Allele/Genotype</th>
<th>Patients</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1345365</td>
<td>N(%)</td>
<td>N(%)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>8 (40)</td>
<td>9 (45)</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>9 (45)</td>
<td>9 (45)</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>2 (10)</td>
<td>1 (5)</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>1 (5)</td>
<td>1 (5)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

In which allele “A” represented 40 % in patients and 45% in healthy individuals ; while allele “G” genotyped with same percentage for both patients and healthy individuals ; on other hand, combined allelic genotypes of “AA” showed 10% , 5% for patients and healthy persons in successive. “AG” registred with 1% for both study groups , while “GG” allelic genotype typed with 0%.Our results are similar to results of (10, 13) in which it seems that ELMO1 plays an important role in the development of type 1 diabetic nephropathy (11) which agrees to the results of our study.

4. Conclusion

We concluded from our study that rs7903146 polymorphism was genotyped in patients with diabetes mellitus in Iraq.

Author’s notes

The manuscript has written by Endnote X7.
5. References


**Author’s notes**

The manuscript of paper was done by Endnote X7.8.